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LETTERS AND CORRESPONDENCE

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Disseminated Histoplasmosis Following Prolonged Low-Dose Methotrexate Therapy

To the Editor: A 43-year-old woman from central Minnesota presented to her primary internist with a 4-month history of weight loss and abdominal distension and a 2-week history of fevers, sweats, weakness, and anorexia. She was receiving treatment with low-dose methotrexate (15 mg every week) for the last 5 years for rheumatoid arthritis. Splenomegaly was noted on examination. Her hemoglobin was 11.5 g/dl, leukocyte count (WBC) was 3.2×10^9/l, and platelets were 115×10^9/l. Routine liver and renal function tests were normal. Bone marrow biopsy showed normocellular marrow with normal trilineage hematopoiesis. A diagnosis of felty's syndrome was made, and treatment with prednisone, 60 mg per day, was started. Her symptoms continued to progress. She was referred to the University of Minnesota for a second opinion 2 weeks later. On examination weight loss, splenomegaly (25 cm below the costal margin), and ascites was noted. Her pancytopenia had progressed: hemoglobin, 8.2 g/dl; WBC, 1.8×10^9/l (56% neutrophils); platelets, 49×10^9/l; and reticulocyte count, 10%. Serum albumin was 2.1 g/dl, bilirubin 2.2 mg/dl, AST 81 ($n = 0$–50 U/l), ALT 44 ($n = 0$–50 U/l), alkaline phosphatase 117 ($n =$ 50–120 U/l), creatinine 0.9 mg/dl, and lactate dehydrogenase 1110 ($n =$ 275–645 U/l). Abdominal CT scan confirmed massive splenomegaly and ascites without hepatomegaly or adenopathy. Over the next 6 days, her hemoglobin further dropped and evidence of disseminated intravascular coagulation appeared. Bone marrow examination showed multiple granulomata and numerous intracellular yeastlike organisms within the macrophages suggestive of Histoplasma (Fig. 1).

Intravenous amphotericin B was begun, and splenectomy was performed the next day. Histopathological examination of spleen, lymph glands from the splenic hilum, and an intra-operative liver biopsy showed extensive granulomata with intracellular yeastlike inclusions. Cultures of the biopsied material yielded *Histoplasma capsulalum.* A test for human immunodeficiency virus (HIV) antibodies was negative. The patient received a total of 2 g of amphotericin B as an outpatient and made an uneventful recovery.

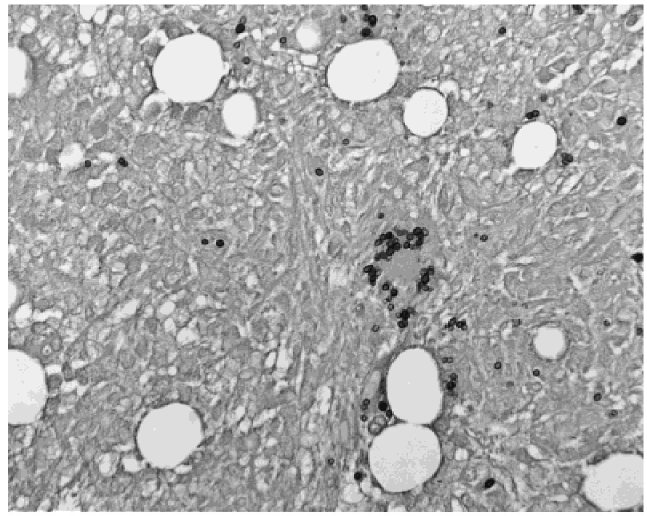

Fig. 1. High magnification (480×) photomicrograph of the bone marrow biopsy showing granuloma with intracellular yeast-like inclusions suggestive of Histoplasma.

Low-dose methotrexate therapy is used for the treatment of a number of conditions including rheumatoid arthritis [1], psoriasis, and bronchial asthma. The risk of infectious complications with such treatment has generally not been emphasized in the literature [2]. Nonetheless, cases of opportunistic infections [3] and immunological abnormalities [4,5] related to the use of low-dose methotrexate have been reported. *H. capsulatum* is a dimorphic fungus that ordinarily causes a self-limiting or slowly progressive chronic infection. In immunocompromised patients, however, a severe progressive illness with extrapulmonary dissemination of infection may result. In our patient, methotrexate therapy is the only immunosuppressive agent we have found that could have predisposed her to develop disseminated histoplasmosis. This case serves as a reminder that patients on low-dose methotrexate can be immunocompromised to a clinically significant degree. A high index of suspicion for opportunistic infections should be maintained when these patients develop fever or constitutional symptoms. If this treatment modality continued to gain popularity—and particularly, if it is used for longer periods of time—more cases of opportunistic infections will likely be seen in future.

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Antiphospholipid Syndrome During Chronic Myelomonocytic Leukemia

To the Editor: Antiphospholipid antibodies, i.e., the lupus anticoagulant and anticardiolipin antibodies are associated with thrombotic manifestations. Defined as the antiphospholipid syndrome [1], it may be primary or secondary to many medical conditions such as systemic lupus erythematosus, solid tumors, or infectious diseases [2]. However, there are few case reports that link antiphospholipid antibodies to the appearance of thrombotic manifestations in patients with leukemia [3,4], and no case has been reported with myelodysplasia. We describe here a case of APS occurring during the course of a chronic myelomonocytic leukemia.

A 69-year-old man presented in March 1995 with macrocytic anemia, and a diagnosis of refractory anemia was made. In October 1995, the anemia worsened, splenomegaly appeared, and a treatment by danazol 600 mg/day was started. In December 1995, blood counts showed leucocytosis (15×10^9/l) with monocytosis (4.3×10^9/l) and bone marrow aspirate showed myelodysplastic features with 15% monocytes, confirming the diagnosis of chronic myelodysplasia. At this time, coagulation tests revealed prothrombin time of 100%, and activated partial thromboplastin time (APTT) of 35 s (control: 30 s). In January 1996, the patient was admitted with increasing shortness of breath caused by bilateral pleural effusions. Physical examination showed marked splenomegaly. An echocardiogram confirmed the presence of pericardial effusions and a diagnostic pleural aspirate revealed a sterile monocytic exudate. Full blood count showed white blood cells (WBC) 73×10^9/l with 42% monocytes. Effusions were tapped and the patient was treated with furosemide and hydroxyurea (2 g daily). After two weeks, his general condition improved, the pleural and pericardial effusions had resolved and the WBC had fallen to 20×10^9/l. At this time the APTT was long (APTT 55 s, control 33 s). The serum contained lupus anticoagulant but no anticoagulant antibodies. In February 1996, the patient developed acute dyspnea and chest pain related to pulmonary embolism confirmed by perfusion scintigraphy. APTT was always prolonged (APTT 60 s, control 33 s) and lupus anticoagulant persisted in the serum. Heparin treatment was administered with a good clinical result. In March 1996, the patient complained of an acute abdominal pain with a massive splenomegaly. Ultrasonography and computed tomography showed multiple, poorly marginated, low-density, heterogeneous lesions, suggesting multiple infarctus or splenic abscess. The patient underwent splenectomy. Histology showed splenic interstitial myelomonocytic infiltration and multiple infarctions. After splenectomy, WBC remained elevated at 15×10^9/l with monocytosis (4.3×10^9/l) and was stabilized with 6-mercaptopurine. APTT was again long (APTT 52 s, control 33 s). The patient died in December 1996 from sepsis.

Although patients with hematologic malignancies frequently develop high titers of anticoagulant antibodies, the clinical significance of this laboratory abnormality is unknown, and only a few case reports have described thrombotic disorders associated with antiphospholipid syndrome during the course of leukemia [3,4]. We believe that in our patient a relationship between thrombosis and antiphospholipid antibodies is probable because APTT was normal at the beginning of the disease and became abnormal later during the course of the disease. Moreover, the patient had no thrombotic events before and the thrombotic episodes occurred only during worsening of leukemia. In patients with chronic myelodysplasia or other hemopathies—which are more associated with hemorrhagic complications than thrombotic events—the occurrence of ischemic events should arouse the suspicion of antiphospholipid syndrome, and antiphospholipid antibodies should be sought.

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Hyperacute Graft-Versus-Host Disease and NKT Cells

To the Editor: A 40-year-old male with chronic myelogenous leukemia in accelerated phase was transferred to our hospital for bone marrow transplantation (BMT). After the first transplantation from his HLA-identical sister, graft rejection occurred, and on day 45 he received a peripheral blood stem cell transplantation (PBSCT) from the same donor using a weak conditioning regimen and immunosuppressive agents. On day 6 after the second transplantation, hyperacute GVHD developed, which consisted of high fever, generalized erythroderma, hyperbilirubinemia, and watery diarrhea. The biopsy specimen of the skin lesion showed a perivascular infiltration of lymphoid cells, which was compatible with hyperacute GVHD [1]. At that time, marked lymphocytosis appeared and reached its highest level, 2.8×10^9/l on day 18. Flow cytometric analysis revealed that the majority of the lymphocyte were $CD3^+$ cells; 66% of these cells were $CD3^+CD8^+CD56^-$ cells, and 28% were $CD3^+CD8^+CD56^+$ cells, the latter of which were classified as NKT cells. Fas-ligand was not expressed on these cells. After initiation of the steroid pulse therapy, hyperacute GVHD improved except for the cholestatic liver injury. He finally died of invasive pulmonary aspergillosis on day 50 after the second transplantation.

Hyperacute GVHD is a severe form of acute GVHD, which occurs 7–14 days after transplantation, typically more than a few days before engraftment [2]. Deleting the posttransplant immunosuppression is associated with frequent and severe hyperacute GVHD [2], but with the widespread use of post-transplant immunosuppression, its incidence has been reduced. At present, the second transplantation after graft rejection is a risk factor of acute or hyperacute GVHD [3]. Because its onset is too early for the donor cells to engraft, the circulating donor cells in peripheral blood are considered to induce it. Thus, it will represent a distinct clinical entity from the usual "acute GVHD". However, because of its low incidence, its pathogenesis, risk factors, and adequate treatments have not been clarified.

This case showed a typical clinical course for hyperacute GVHD. Interestingly, there was a marked correlation between the severity of hyperacute GVHD and the number of peripheral lymphocytes. We found that when the

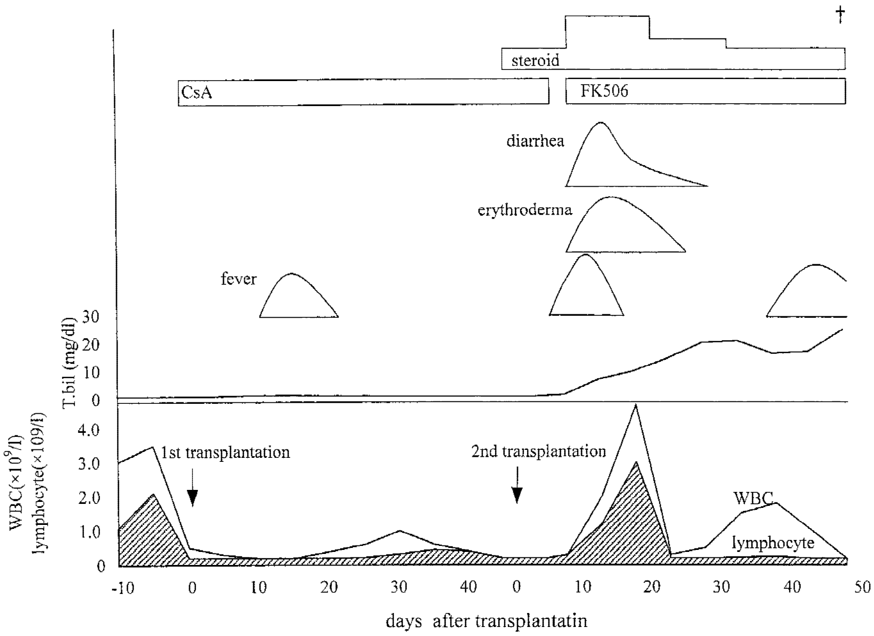

Fig. 1. Clinical course of graft rejection and hyperacute GVHD.

number of the lymphocytes was at the highest peak level, 28% of them comprised NKT cells ($CD3^+CD8^+CD56^+$ cells), which represent a minor lymphocyte population in normal peripheral blood in human. Although NKT cells have a higher cytotoxic activity [4], it remains unknown what roles they play in the immune system and the development of diseases. Our case might show a close association between hyperacute GVHD and NKT cells. Considering the immunological characteristics of NKT cells, there is a possibility that those cells could cause a severe hyperacute GVHD, which is different from ordinary acute GVHD. To our knowledge, this is the first report that describes the association between NKT cells and any types of GVHD, including hyperacute GVHD.

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Protease Inhibitors and Haptoglobin for Treatment of Renal Failure in Paroxysmal Nocturnal Hemoglobinuria

To the Editor: The clinical course of many patients with paroxysmal nocturnal hemoglobulinuria (PNH) is punctuated by episodes of increased hemolysis. Not infrequently, these episodes are initiated by infections, viral or bacterial. The episode is characterized by darker urine, which remains markedly hemogrobinuria throughout the day. Patients often complain of malaise and fatigue and may have abdominal pain [1,2]. A major complication of a hemolytic episode is acute renal failure (ARF) [3]. This ARF often needs to be treated with hemodialysis and a large amount of hydration. We report that antienzyme and haptoglobin produced an improvement from the severe renal dysfunction of the hemolytic crisis without hemodialysis.

The patient is a 28-year-old man who had been diagnosed as having PNH 6 years ago. At this time, he had undergone hemodialysis in several times for ARF associated with hemolytic crisis. After his discharge, he had stopped going to the hospital. He was admitted to our hospital for fever and fatigue. The examination suggested hemolytic anemia, and hemosiderinuria and severe renal dysfunction were noticed. Laboratory data were as follows: hemoglobin, 8.4 g/dl; reticulocyte, 31%; white blood cell count, 5,000/μl; and platelet count, $15.2 \times 10^4/\mu l$. Sucrose hemolysis test and acidified-serum lysis test (Ham test) were both positive. Respectively, positive rates of decay accelerating factor (DAF) and CD59 were 38.8% (control 100%) and 89.9% (control 100%). Thus the preliminary diagnosis was confirmed. Blood chemistry results were as follows: GOT, 143 IU/l; GPT, 19 IU/l; lactic dehydrogenase (LDH), 5973 IU/l (normal range 132-526 IU/l); LDH isozyme, LDH1, 36.8%; LDH2, 39.7%; total bilirubin, 1.96 mg/dl; direct bilirubin, 0.58 mg/dl; blood urea nitrogen, 39 mg/dl; and serum creatinine, 4.41 mg/dl. Serum haptoglobin level was less than 10 mg/dl (not detected).

Immediately after admission, he was treated with aggressive intravenous hydration and administration of ulinastatin and haptoglobin. Ulinastatin was given in doses of 50,000 IU three times daily, and haptoglobin was given in doses of 4,000 IU by continuous infusion. His symptoms disappeared and laboratory data returned to normal in about two weeks (showed

TABLE I.

Tests		Treatment Before	After
WBC	[/μl]	5,100	4,400
RBC	[$\times 10^4$/μl]	320	435
Hgb	[g/dl]	7.7	11.0
Hct	[%]	24.9	35.2
Plt	[$\times 10^4$/μl]	15.5	22.1
Reticulocyte	[‰]	31	34
T-Bil	[mg/dl]	1.96	0.81
D-Bil	[mg/dl]	0.58	0.22
GOT	[IU/l]	66	42
GPT	[IU/l]	17	51
LDH	[IU/l]	5,973	1,295
BUN	[mg/dl]	50	16
Creatinine	[mg/dl]	6.52	0.86
CRP	[mg/dl]	6.3	0.3
Haptoglobin	[mg/dl]	≤10	135
Urine examination			
Occult blood		3+	–
Protein	[mg/dl]	50	–
Casts	[×400]	–	–
Hemosiderin		+	–

Table I). Following treatment of his renal failure, he made progress and was discharged 1 month later. Then he had hemolytic attack several times after infection. In the outpatient department of our hospital, he was given intravenous hydration, haptoglobin, and protease inhibitor (gabexate mesilate and ulinastatin). There was a striking clinical improvement each time until he no longer needed to be readmitted for hemolytic crisis.

PNH is an unacquired chronic disorder of the hematopoietic stem cell characterized by chronic or acute intravascular hemolysis caused by increased erythrocyte sensitivity to activated complement components [1]. This sensitivity is attributed to the absence of a component-regulating protein, DAF, or a number of other proteins that are missing from the membrane of the abnormal red cell [2]. Despite the remarkable progress in our understanding of this disorder, treatment has remained largely supportive. Venous thrombosis is one of the severe complications of PNH, requiring anti-coagulation. It has been regarded that ARF of PNH is caused by dysfunction of tubular cells because of the deposition of hemosiderin and microvascular thrombosis [3]. The liberation of thromboplastic material from hemolyzed red cells has been blamed for the increased tendency to thrombosis that characterizes PNH. In addition, intravascular coagulation may be triggered by the interaction of complement components with complement-sensitive PNH platelets, or vascular occlusion may result from collection of stroma derived from broken-down red cells. Gabexate mesilate is known to be a kind of serine protease inhibitor. Serine protease is an important factor in the activation of complement and the coagulation system. Serine protease inhibitor is known to be a factor Xa antagonist and inhibitor [4]. It is possible that protease inhibitor control the activation of the complement system. Ulinastatin is a Kunitz type protease inhibitor, and it was reported that ulinastatin has a protective effect against certain drugs nephrotoxicity, and its prevention of the increase in lysosomal fragility is probable mechanism involved in the renal protection [5]. Haptoglobin is a serum glycoprotein which has the ability to bind irreversibly free hemoglobin released into the circulation. It may prevent renal tubular cells from accumulating of hemosiderin [3]. Furthermore, it is a well-known fact that extracorporeal circulation is burden on circulatory organs therefore hemodialysis must not be used if this can be avoided. Our treatment, on the other hand, has produced a improvement in renal function and hemolytic factor without hemodialysis. It did not show any side effect each time. Hereafter, we will follow up this patient carefully and make further examinations.

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